REMARKS

The claims in the case are claims 1-11. Claims 1 and 11 are the independent claims. In its broadest sense, each of these independent method claims relates to lowering egg cholesterol levels of laying fowl by administering polycosanol. The Examiner has rejected each of these independent claims on a theory of inherency and lack of novelty, based upon three references. Those references, Havens, Lane et al., and JP4-210560 each teach feeding laying fowl a diet that contains something which the Examiner urges has within it polycosanol. Havens teaches cabbage; Katta teaches rice bran; and, Lane teaches rice bran. From this, the Examiner leaps to the conclusion that in each instance the independent claims are anticipated because the compositions containing cabbage and/or rice bran would inherently lower cholesterol as they contain some amount of polycosanol. This conclusion, reached by the Examiner, on nothing more than a leap of faith is simply wrong. Each of the claims in independent form, i.e. 1 and 11, require "administering a cholesterol lowering effective amount". The Examiner relies on nothing but faith to urge that this claim limit is met. Respectfully, it is not; and therefore the references cannot anticipate. A discussion of each reference and its true teaching follows.

JP4-210560A Yamamoto. The Examiner states that the inventor in E1 claims rice bran as an agent for lowering egg yolk cholesterol and since dried bran contains wax esters, a source of polycosanol, it anticipates use of polycosanol to change the cholesterol content of yolk. With respect, a careful reading shows that the inventor did not use rice bran but <u>fermented</u> rice bran to produce an "intermediate feed" that was fed to chickens. Since the starting rice bran has been altered by fermentation, it is incorrect to conclude that there is any polycosanol in the intermediate feed.

US 5,091,195 Havens. Similar to above, the Examiner states that since cabbage contains polycosanol then it would inherently use polycosanol for the laying hen diet to lower cholesterol. However, the inventor teaches away from this assertion by stating in col. 2, lines 19-32 that a component in cabbage induces the production of cytochrome P-450 that "detoxifies" cholesterol and allows it to be used for energy purposes, resulting in less cholesterol for the egg. This mechanism is specifically claimed in his Claim 5.

US 5,578,584 Katta et al. With respect, the Examiner has misunderstood the role of rice bran in Table 10 of the above-referenced patent. The feed described in Table 10 is a basal feed that contains rice bran that was added to two groups of egg laying hens. One group received only the basal feed (placebo, col. 10, lines 23-42) and the other group received the basal feed plus galacto-oligosaccharides (col. 10, lines 44-4), the compounds that were the subject of the invention. The effect of the galacto-oligosaccharides was to lower egg yolk cholesterol. If the inventor thought rice bran had an effect on cholesterol lowering, he would not have used it as a basal feed. Once again this teaches away from the use of polycosanols as agents for lowering egg yolk cholesterol.

US 6,239,171 Lane et al. The purpose of this invention was to demonstrate that certain tocotrienols and tocotrienol-like compounds found in rice bran or its oil lower serum cholesterol and also can be used for the treatment of inflammatory conditions. Example 8 clearly shows that the wax fraction, which contains the polycosanols and phytosterols, do not plan a role in cholesterol reduction. Thus, rice bran wax (Protocol VII, col. 22, lines 57-62) was isolated and added to a placebo basal chick diet (Table IV, entry 1) to determine its effects on circulating cholesterol. The inventor concluded (col. 31, lines 25-26) that the wax fraction that contained the sterols (and the polycosanols) had no effect on circulating cholesterol.

The Examiner should also be aware that fiber has an impact on the cholesterol content of eggs. McNaughton reported that egg yolk cholesterol was significantly decreased when fiber from alfalfa meal, oats, sunflower meal, rice mill feed or wood shavings was fed to laying hens ("Effect of dietary fiber on egg yolk, liver, and plasma cholesterol concentrations of the laying hen, *J. Nutr.* 108:1842-1848, 1978 (copy enclosed)). The consumption of large amounts of cabbage would also be expected to be a good source of fiber. In summary, when gross diet feeding studies are performed which contain a source of fiber, attribution of a cholesterol lowering effect to one component is problematic unless that component is purified and added to the laying hen in a controlled trial.

There is simply no basis to conclude that these references properly interpreted in fact use polycosanols to lower cholesterol by administering an effective amount to lower cholesterol. Inherency must be based on evidence and facts not upon wild speculation. Purdue Pharma L.P.
V.Faulding Inc., 56 U.S.P.Q.2d 1481, 1483 (Fed. Cir. 2000) (claim limitation is inherent if one skilled in the art can immediately discern the limitation at issue) (emphasis added); Tronzo v.Biomet, Inc., 47 U.S.P.Q.2d 1829, 1834 (Fed. Cir. 1998) ("In order for a disclosure to be inherent ... the missing descriptive matter must necessarily be present in the parent application's specification such that one skilled in the art would recognize such a disclosure.") (emphasis added); and Silvestri v. Grant, 181 U.S.P.Q. 706, 709 (1974) (inherent properties are redundant and add no additional limitations beyond those recited) (emphasis added).

As the recent Supreme Court KSR case teaches, references that teach away can be evidence of patentability. Here the Examiner cites references when taken together teach away from the use of polycosanols and the waxes from which they are derived for lowering circulating cholesterol and the cholesterol content of egg yolk. In this way it cannot be seen to be

anticipated (it's not inherent as demonstrated in the discussion above) and neither can it be obvious since the references teach directly contrary to the claimed process. Reconsideration and allowance is requested.

No fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

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Effect of Dietary Fiber on Egg Yolk, Liver, and Plasma Cholesterol Concentrations of the Laving Hen

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ABSTRACT Two experiments were conducted to determine the effect of dietary fiber sources and level on egg yolk, liver, and plasma cholesterol concentrations of White Loghorn laying hem. Initially, dietary fiber with the concentration of White Loghorn laying hem. Initially, dietary fiber with the concentration of White Loghorn laying hem. Initially, dietary fiber with the concentration of the concentration

INDEXING KEY WORDS dietary fiber · cholesterol concentration laying bens

Dietary fiber has been implicated in reent years as causing a reduction in serum and body cholesterol. Fiber, the non-digestible component of a diet, has been referred to as a natural hypocholesteremic agent. Ralman and Zilmanni 11 cm. reserved to as a natural hypocholesteremic agent. Balmer and Zilversmit (1) found that condigestible components of an animal's diet have major influences on both plasma cholesterol concentrations and turnover, as well as feeal excretion.

Increasing dietary fiber has been shown to significantly decrease serum cholesterol and/or artery deposition of plaque in humans (2), rabbits (3), rat (4), chicks (5-6), turkeys (7), and laying hens (8-9). Efforts have been made by numerous re-searchers to lower egg yolk cholesterol. A few of these areas of research include drugs (10), dietary proteins (11), energy level or energy sources (12), vitamin A (13), ascorbic acid (14), and vanadium Diet and Barnet (18) found there alcale.

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Turk and Barnett (16) found that alfalfa, when added to a corn-soy laying hen diet, was the most effective of the fiber sources tested for decreasing egg cholesterol with the least loss of egg size, feed efficiency, and egg production, while cellulose only

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stightly reduced egg cholesterol. Menge et al. (8) found that increasing the dietary fiber level from 4.1 to 17.7% with cellulose caused a reduction in serum cholesterol and an increase in egg yelk cholesterol. Soliton and the strain of the series of the

Husseini et al. (9).

Fiber has been found to reduce body cholesterol of various animal species. However, relationships of dietary fiber to edible excretory products, such as eggs, have not

been tested thoroughly with the exception of extremely high dietary fiber levels, which have been shown to affect production of laying hens. In studies that have been conducted, both hype- and hypercholesterenic a gents were added, thus multiplications of the studies of the s

PROCEDURE

A total of 702 White Leghorn hoas were randomly placed in wire eages when they were 31 weeks old in experiment 2. Sixteen lots with a total of 576 kirds in experiment 1 and 18 lots with a total of 216 pirth in experiment 2 were fed experimental diets for 140 and 58 days, respectively. Foru lots of 36 birds each in experiment 1 and three

TABLE 1 Composition of diets in experiment 1

	Orude fiber, %					
Ingredient	2.051	4.41	6.68	8.79		
	%	%	%	%		
Yellow corn	69.65	63.45	57.63	54,71		
ovbean meal, 49% protein (N × 6.25)	17,94	11.90	5.98			
loybean meal, 49% protein (N × 6.25) lunflower meal, 33% protein (N × 6.25)		10.63	20.88	30.07		
nimal fat (tallow)	1.49	3.37 2.43	5.00 2.37	5.00		
Dicalcium phosphate (22% Ca, 18.5% P)	2.49	2.43	2.37	2.31		
imestone	7.52	7.32	7.23	6.98		
odlum obloride	0.25	0.25	0.25	0.25		
race element premix*	0.25	0.25	0.25	0.25		
dethionine hydroxy analogue—Ca. 93%	0.34 0.07	0.30	0.27	0.24		
dysine-HCl (98%)	0.07	0.10	0.14	0.19		
Total	100.00	100.00	100.00	100.00		
calculated analysis						
Crude protein. %	15	15	15	1.5		
Metabolizable energy, keal/kg	2970	2970	2970	2970		
Total calcium, %	3.50	3,50	3,50	2,50		
Available phosphorus, %*	0.55	0.55	0.55	0.55		
Meth. & Cvat %	0,80	0.80	0.90	0.80		
Lysins, %	0.80	0.80	0.80	0.80		
herpical analysis						
Total fat. %	4.34	6,11	7.66	7.75		
Crude fiber, %	2.05	4.41	6.68	8.79		
Cholesterol, mg/g	0.47	0.79	1.22	1.26		

³ Standard laying hen diet. ⁴ The laying hen premir furnished the following amounts of other ingredients per kilogram of feed retirity plannitate, gealand constend, 717 UU rebolestiries, 2020 ICU; visiamin E, 1.1 UU; rebollsvin, 3-3 mg; rikedn, 22.0 mg; d-pautobhenic seld, 6.0 mg; vitiamin B-12, 5.5 mg; obsidered to be 30% available to the chicking, copper childred, 2 mg. ⁴Posphoric from plans sources is considered to be 30% available to the chicking.

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lots of 12 each in experiment 2 were fed each experimental diet. Water and feed were given to all birds all thitum throughout the test periods. Experimental diet 5 (constant) and the second of the s

or exceed National Research Council's (19) recommendations for poultry. Natural feed ingradients such as corn, soybean meal, and animal fat were used to formation and animal fat were used to formation of the country industry. Feed samples were ether-extracted and deterary fat and cholestered determinations made (See tables 1 and 5). Frontinate analysis of fiber sources were determined by the procedures of Associations of the country of t

TABLE 5

	Composition of dists in Experiment 2								
	Source of added fiber								
Ingredient	Bessl	Alfalfa mosi	Ground whole oats	Sunflower	Rice mill	Wood			
Yellow corn	72.71	66.12	65.25	70.17	63.14	66.79			
Soybeen meal 49% protein (N × 6.25)	17.55	16.10	16.33	12.73	18,51	18,61			
Alfalfa meel, 17% protein (N × 6.25) Ground whole cata	=	7.69	18.18	=	=	=			
Sandama mari				8.00	_	_			
25% protein (N × 6.25) Rise mill feed, _6% protein (N × 6.25)	_	_	_	_	6.25	3.83			
Wood shavings Animal fet (Tallow)	=	1.08	1.00	=	2.81	1.55			
Dicalolum phosphate (22% Ca., 18.5% P) Americae	1.94 7.18	1.97	1.94 6.70	1.92 6.58	2.02 6.65	1.94 7.18			
iodium chloride Prace element premixi	0.25	0.25	0.25 0.25	0.25 0.25	0.25 0.25	0.25 0.25			
fethionine hydroxy	0,10	0,10	0,10	0.08	0.12	0.10			
lysine HCl (98%)	100.00	100.00	100.00	0.04 100.00	100.00	100.00			
alculated Analysis Crede protein, % Metabolisable marry.	15.00	15.00	15.00	15.00	15.00	15.00			
load /los	2919.00 3,25	2850.00 3.25	2858,00 3,25	2878.00 3.25	2960.00 3.25	2862.00 3.25			
Total calcium, % Available phosphorus, % Methionine + Cystine, %	0.45	0.45	0.45 0.50	0.45 0.60	0.45	0.45 0.60			
Lysine, %	0.75	0.75	0.75	0.75	0.75	0.75			
Total fat. %	2.96	8.92 3.90	4.11 8.68	2.87 3.90	5.49 3.91	4.52 4.01			
Crude fiber, % Cholesterol, mg/g	0.47	1,64	0.90	0.49	0.69	0.40			

¹The layer premix furnished the following amounts of oth almitates, gaistin coated, 7717 IU; sholesaloiferd, 2205 IC sain, 23.0 mg, d-paentoheule said, 6.5 mg, vitania B-12, 5. 5 mg; mangasses, 68.3 mg; sine, 44 mg; lodine, 1.25 mg; Phosphorus from plant sources is considered to be 30% availa-

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TABLE 2
Plasma and liver choissierol and plasma triglycerides of hone fed

-	Pla	ema	Liver		
Crude fiber	Cholesterol	Triglycerides	Cholesterol	Weight	
%	mg/100 ml		mg/a	g	
2.05 4.49 6.68 8.79	158.33 ±7.21*.4 184.66 ±7.54* 185.00 ±9.01* 178.33 ±8.69*	297,65±12,95* 293,63±15,44* 272,65±14,13* 245,23±11,49*	2.52±0.114 2.68±0.144 3.04±0.213 8.38±0.089	40.01 ± 2.53° 42.95 ± 1.84° 44.54 ± 2.51° 45.61 ± 2.25°	

 $^{^1}$ Means within a column and without a common superscript letter are significantly different (P < 0.05). Mean means for 30 hers.

days in each experiment during the last week of the test. Blood was collected from a total of 20 hears in experiment 1 and 15 hears in experiment 2 for each test group. Plasma cholesterol concentrations were determined by using the Lebermann-Burchard reagent of consisting of suffuring the control of the c

(21) and Witter and Whitner (22).
On 3 consecutive days seach week, during the last 4 weeks of each experiment, 15 eggs weighting 50 ± 2 g and 10 eggs weight of 50 ± 2 g and 10 eggs weighting 50 ± 2 g and 10 eggs weighting 50 ± 2 eggs from each group were broken and yolks were cleaned thoroughly with cheese-cloth. All yolks from each test group were pooled and yolk cholesterol determined. Folks samples were extracted by the method yolks are good to the contract of the contr

days in each experiment during the last of Folch et al. (23). Yolk cholesterol was week of the test. Blood was collected from determined with the use of the Lieber-

determined with the use of the Liceormann-Buchard reagent.^{1,2} Shinistical examination of the data was performed using the analysis of variance (24). Duncan's New Multiple Range Test (25) was used to determine significant disferences between means. All statements of significance refer to the 5% level of probability.

RESULTS AND DISCUSSION

Experiment I. Results of experiment 1 are shown in tables 2 to 4. No significant differences in plasma cholesterol (table 2) were found due to dietary fiber level. How-

*Hynel, Inc. P.O. Box 36129, Honsten, Tenza.

*Mention of a trade name, proprietary product, or
pecide equipment does not concilitate a guarantee or
warranty by the U.S. period to the exclusion of other
products that may be unitable.

TABLE 3

It recipit and see welk cholesterel concentrations of here receiving various crude fiber levels, experiment 1

		Yelk	rt.		Yolk oho	lestarol		Yolk obei	mterel
	Xec :	Th., 2		Egg	rt., g		Eqs :	rk, g	
Crude	50±2	64 ±2	Monal	89 ±2	64.42	Mean!	59 ±3	64;±3	Meen;
74		•			Me.	re .		mg	
2.05	19.00	21.02	20.05 ± 1.06*	14.42	93.93	14.17 ±0.70*	275.28	292.54	\$83.88 ±15.84
4.41	19.10	20.37	19.74 40.84	12.75	13.75	12.75 40.644	262.68	280.15	271.41 ±13.33
0.66	18.46	30.64	19.56 +0.60	12.53	12.83	13.33 ± .504	236.85	271.98	284.40 ±14.51
5.79	18.66	20.20	19.48 ±1.13	12.33	12.92	12.62 ±0.75+	230,14	262.19	248.16 ±16.55
Manni	18.636	20.24		12.33+	13.60-		251.73	276.71	

¹ Means within a column or rew grouping and without a common supersoript letter are significabily different (P < 0.05). Mean man, 1 No significant difference (P < 0.03) in yolk weights were found due to distant treatment. Mean masses. ■ Eags of a column paint is two most of the delayed a constraint in destruction.

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TABLE 4 Effect of fiber level on egg production and weight, experiment I

	Test results					
Crede Sher	Hen-day lay	Avg. egg wt.	Feed consumed			
5	5		g/han/day			
2.05	78.26 +3.90*	59.4 ±2.51*	78-19 m3-01*			
4.41	77.08 -4.05	54.4 ± 1.80	87.63 止 8.95			
6.65	77.08 ± 2.13	59.3±2.30*	91.35±2.41el			
8.79	77.17 ±3.15*	59.1 ±1.97*	95.65 ±8.25			

1 Money within a column and without a common supersuring letter are signifi

ever, plasma triglycarides decreased as ever, plasma triglycarides decreased as hens were fed diete with increasing dietary fiber levels (r = -0.95). Although total dietary fat (bible 1) increased as dietary fiber increased in order to equate metalolizable energy among diets, plasma triglycerides decreased as dietary fat and fiber increased (see table 5) as the fiber increased (see table 5) except for the fiber increased (see table 5) as both fiber and animal fat increased it laying ben diets. Either fiber or animal fat might have caused the increased liver weights. meet or animal fat might have caused the increased liver weights. No conclusion was reached as to the cause of liver weight in-creases, since liver lipids were not deter-mined.

Egg weights were separated into two distinct egg weight groups weighing 59 ± 2 or 64 ± 2 g and egg yolk cholesterol was z or os = z g and egg your consestered was determined on each group. Eggs of similar weights were taken in order to delete any possible interaction of egg weight and yolk cholesterol. Yolk cholesterol and

weights are shown on table 3. Egg yolk weights numerically decreased as fiber was increased in laying hen diets; however, these values were not significantly different

terean:
Milligrams yolk cholesterol per g of yolk
and mg cholesterol per yolk decreased as
bens were fed increasing dietary fiber
levels. Total cholesterol per yolk decreased
4.39, 10.38, and 13.39% by feeding crude
dietary fiber levels of 4.41, 6.68, and 8.79%. respectively, to hens as compared to a com-soybean meal basal diet containing 2.05% crude fiber. Although dietary fat increased as dietary fiber increased (table

increased as decary noer increased (table 1), the condusion is reached that only dietary fiber influenced yolk cholesterol, since Miller and Katsoulis (28) found no significant differences in either blood serum or egg yolk cholesterol concentrations with increases of the content of the cont

or egg yolk cholesterol concentrations with increasing dietary animal fat. No significant differences in either egg production or egg weight (table 4) were found due to diet variation. However, feed consumption increased as dietary fiber in-

commuption increased as dietary fiber in-creased in the diet of laying hous.

Experiment 2, Results of experiment 2 are shown in tables 6 to 8, Plasma cholesterol (see table 6) was significantly lower when laying hens were fed diets containing a significant of the significant of the significant of the significant of the significant in the significant was fed to have diet containing oats were fed to laying heas as compared to laying heas diets containing oats were fed to laying heas as compared to laying heas of the containing oats were fed to laying hease of the significant differences in yolk weights (table 7) were found among diets fed.

Plasma and liver cholesterol and plasma triglycerides of here fed various fiber sources, experiment 2

	Plass	ns.	Liver		
Fiber source	Cholesterol	Triglycerides	Cholesterol	lesterol Weight	
	mg/100	2 ml	mg/g		
None Alfalfa meal Ground whole oats Sunflower meal Rice mill feed	128.97 ± 10.401.4 55.98 ± 8.53° 87.99 ± 9.14* 141.03 ± 11.594 98.20 ± 10.126 47.78 ± 7.25*	319.41±14.13°* 346.80±18.55** 873.49±21.31° 325.57±10.21°* 309.76±12.14° 326.70±16.91°*	1.65±0.06* 1.94±0.06* 1.71±0.11* 1.60±0.06* 1.78±0.12* 1.56±0.10*	44,07 ±2.21* 45.01 ±2.35* 46.34 ±1.94* 56.24 ±2.29* 52.09 ±2.61* 53.76 ±1.99*	

I Means within a column and without a common superscript letter are significantly different (P < 0.05). Mean ± 35M for 15 beas.

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TABLE 7

		Yolk	Yolk wi.		Yolk cholesterol			Yolk cholestard		
	Ees '	PL. 5		East	W- 8°		Per	wh. #		
Fiber source	50 ±2	04 +2	Meant	E9 ±2	64.12	Maant	50 ±1	64.±2	Mean	
						=9/0		Mg.	,	
Nome Alfalfa meal Greund whole cats Sunflower meal Rice mill feed Wood shavings	18.22 18.00 18.00 18.31 18.40 18.30	20.12 20.01 19.85 19.60 20.09 20.04	19.17 ±0.55 19.10±1.15 18.97 ±0.94 18.90 ±0.46 19.39 ±1.06 19.17 ±1.11	13.93 13.06 11.95 12.79 12.78 11.96	14.78 18.70 12.06 12.75 13.21 12.79	12.80 ±0.84 13.52 ±0.71 12.50 ±0.65 12.27 ±0.77 12.09 ±0.94 12.37 ±0.53	235.47 227.76 216.18 234.19 234.26 218.68	295.32 274.15 239.20 208.12 206.44 254.25	265,40 ±14.67 265,95 ±19.51 267,66 ±16.16 251,16 ±14.91 260,45 ±12.00 267,44 ±18.18	
Mean'	18.27	10.04*		12.57	13.55		210.74	269.741		

No rigidions difference (F < 0.05) in yolk weights were found due to dictory treatment. Mean acres. 1 Name of equal veight was used for shelpistept ensentration determinations. 2 Mean within a column or your prosping and videout a comman preparently later as elaphantary different (F < 0.05).</p>

Yolk cholesterol of laying hens was decreased by fseding either oats or wood shavings as fiber sources when compared to either the basal or diets containing alfalfa meal, sunflower meal, or rice mill feed.

fundamental to the control of the co

(18) reported that vegetable oil increased yelk cholesterol, therefore, the depression of yelk cholesterol which might have co-curred with cets in the study of Husseini et al. (9) was probably angated by the addition of vegetable oil. Our results do indicate that our reduced year with the collection of laying hans by about 10% that? "I when compared to a count-toybeam basal

diet.
Milligrams cholesterol per g of liver increased (table 8) significantly when laying house west fod affalia need as compared to heas fod the basal, cats, sunflower meal, rice mill feed, or wood shaving. Total liver cholesterol increased significantly when heas were fed either affalia meal or rios mill feed as compared to hear fed the basal diet.

Egg production and egg weight were not

TABLE 8

Effect of fiber source on egy production and reciphts, experiment 9

	Test results			
Piber source	Hen-day lay	Avg. ogg wt.	Feed consumed	
None Alfalfa meal Ground whole osts Sunflower meal Rice mill feed Wood shavings	% 79.38±4.05* 78.31±3.51* 78.50±3.84* 78.95±4.16* 78.22±4.17* 79.06±3.26*	60.0±3.01° 59.5±2.29° 60.1±2.65° 59.8±2.57° 59.4±2.88° 60.1±2.53°	g/hen/day 80.58±4.55° 87.93±4.21° 89.21±3.89° 88.60±4.05° 89.09±3.63° 86.32±3.51°	

[:] Means within a column and without a common superscript letter are significantly different (P < 0.05). Mean salar for 36 hans,

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